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PATENT TRADEMARK OFFICE



Docket No: 0646/1D205-US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Ramesh A. BHAT; Ruth HENDERSON; Chulai HSIAO;
Sotirios K. KARATHNASIS

Serial No.: 09/429,832

Art Unit: 1646

Confirmation No.:

Filed: October 29, 1999

Examiner: N. Basi

For: NOVEL HUMAN ESTROGEN RECEPTOR-BETA

MARK-UP FOR RESPONSE TO OFFICIAL ACTION UNDER 37 C.F.R. §1.111

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

August 22, 2001

Sir:

IN THE SPECIFICATION

First full paragraph, page 4:

Figure 1 is a schematic illustration of the oligonucleotides used for PCR
amplification of human estrogen receptor- β (hER β) cDNA (SEQ ID NO:5 and SEQ ID
NO:6).

First full paragraph, page 7:

Alignment of the known rat ER β sequence (Kuiper et al., *Proc.Natl.Acad.Sci.USA* **93**:5925, 1996) with that of a human homologue (Mosselman et al., *FEBS Letts.* **392**:49, 1996) suggested that the human sequence lacked at least the ultimate and penultimate residues at its aminotermminus, as shown below:

Rat: MTFYSPAVMNYS . . . (SEQ ID NO:3)

Human: - - GYSPAVMNYS . . . (SEQ ID NO:4)

Based on this information, PCR primers were designed that supplement the human sequence with the two missing aminoterminal residues M and T and with an artificial Kozak translation initiation sequence. The forward primer, having the sequence (SEQ ID NO:5) 5'-GGAAGCTTGTCGACCATCATGACCGGCTATAGCCCTGCTGTGATG-3' and a reverse primer, having the sequence (SEQ ID NO:6) 5'-GGATCTAGAGTCGACGCGTCACTGAGACTGAGGGTTCTGG-3' were used to amplify hER β sequences in a reaction containing the following components: 2 μ l of the cDNA template described above; 1X PCR buffer; 200 μ M of each deoxynucleoside triphosphate, 2 units of hot tab polymerase (Amersham, Arlington Heights IL), and 1 μ g of each of the forward and reverse primers. The reaction mixture was heated to 95°C for 2 minutes, annealed at 52°C for 1 minute, and amplified using 36 cycles of 72°C for 1.5 minutes.

First full paragraph, page 8:

A fragment of approximately 1500 bp in length was produced. The fragment digested with *Hind*III and *Xba*I (which cleave at sites present in the forward and reverse primer sequences, respectively, but not in the main body of the amplified cDNA sequence) and cloned into the corresponding sites of the pcDNA3 expression vector (Invitrogen, Carlsbad CA). This assymetric cloning strategy places the 5' end of hER β cDNA under the control of the viral CMV promoter in pcDNA3 (Figures 1 and 2). Several insert-containing pcDNA3 clones were identified. Plasmid DNA was prepared from three clones using a plasmid purification kit (Qiagen, Santa Clarita CA)

and their insert sequences were determined by the dideoxy termination method. One clone (designated R61010-2.24 or Clone 3) was found to contain an insert with a nucleotide sequence identical to the published hER β sequence (Mosselman et al., *FEBS Letts.* **392**:49, 1996) and had the following 5' end structure:

M T G Y . . . (SEQ ID NO:7)
CCATC ATG ACC GGC TAT . . . (SEQ ID NO:8)

This clone was designated "truncated hER β " or hER β_T .

Second full paragraph, page 8:

To verify the aminoterminal and upstream sequence of human hER β , two independent approaches were taken, as described below.

(1) 10 μ l of a human ovary 5'-Stretch cDNA library (Clontech, Palo Alto CA) was mixed with 50 μ l of 1X K solution (1X PCR Buffer (GIBCO-BRL, Gaithersburg MD), 2.5 mM MgCl₂, 0.5% Tween-20, 100 μ g/ml Proteinase K), and the reaction mixture was incubated at 56°C for 2 hours, then at 99°C for 10 minutes. 5 μ l of this reaction mixture were then used as template in a nested PCR reaction. For the first round, the forward primer (pDR2 sequencing primer, Clontech, Palo Alto CA) had the sequence 5'-CTGGTAAGTTTAGTCTTTTGTGTC-3' (SEQ ID NO:9), and the reverse primer (hER β -specific, designated oligo #12908) had the sequence 5'-GCTTCACACCAAGGACTCTTTTGAG-3' (SEQ ID NO:10). The reaction contained 1X Klentaq PCR reaction buffer (40 mM Tricine-KOH, 15 mM KOAc, 3.5 mM Mg(OAc)₂, 75 μ g/ml bovine serum albumin); 0.2 mM of each dNTP; 0.2 μ M of each of the above primers, and 1 unit of Klentaq Polymerase Mix (Clontech, Palo Alto CA). Touchdown

PCR conditions were as follows: 5 cycles of 94°C for 2 seconds and 72°C for 4 minutes, followed by 30 cycles of 94°C for 2 seconds and 67°C for 3 minutes.

First full paragraph, page 9:

Excess nucleotides and primers were removed from the first round PCR reactions by purification over Wizard PCR columns (Promega, Madison WI). A second-round PCR reaction was performed using 2 μ l of the purified first round reaction mixture. For the second round, the forward primer was the pDR2 sequencing primer shown above, and the reverse primer had the sequence 5'-GTTGGCCACAACACATTTGGGCTTGT-3' (hER β -specific, designated oligo #13871) (SEQ ID NO:11). The PCR reaction and cycling conditions were identical to those employed in the first round. The products were cloned into the pCR2.1 (Invitrogen) and three resulting clones were sequenced. All three clones (designated L1, L2, and L3) contained hER β inserts of different lengths, all of which were homologous to hER β , and to each other.

Second full paragraph, page 9:

(2) A Marathon Ready thymus cDNA kit (Clontech) for 5' rapid amplification of cDNA ends (RACE) was also used to isolated hER β 5' clones. In the first round of a nested PCR reaction, 5 μ l of human thymus Marathon-ready cDNA (Clontech) was used as template. The forward primer had the sequence 5'-CCATCCTAATACGACTCACTATAGGGC-3' (Adaptor primer 1, Clontech) (SEQ ID NO:12), and the reverse primer had the sequence 5'-

GCTTCACACCAAGGACTCTTTTGAG-3' (hER β -specific, designated oligo #12908) (SEQ ID NO:10). The PCR reaction and cycling conditions were identical to those described in (1) above.

First full paragraph, page 10:

Excess nucleotides and primers were removed from the first round PCR reactions by purification over Wizard PCR columns (Promega). A second round PCR reaction was performed using 2 μ l of the purified first round reaction. For the second round, the forward primer had the sequence 5'-ACTCACTATAGGGCTCGAGCGGC-3' (nested adaptor primer 2, Clontech) (SEQ ID NO:13), and the reverse primer had the sequence 5'-GTTGGCCACAACACATTTGGGCTTGT-3' (hER β -specific, designated oligo #13871) (SEQ ID NO:11). The second round PCR reaction and cycling conditions were identical to those employed in the first round. The products were cloned into the pCR2.1 vector and two clones were sequenced. The two clones contain insert sequences of different lengths that are homologous to hER β , to each other, and to the sequences isolated from a human ovary cDNA library as described above.

IN THE CLAIMS

17. (Amended) A purified polypeptide comprising a sequence selected from the group consisting of the sequence depicted in Figure 4, SEQ ID NO:2 [and function-conservative variants thereof].